off-pathway transitions are energetically unfavorable as they are associated with the increase in the neck linker tension.

654-Pos Board B454
A Micromechanical Model of Cargo Transport by Multiple Microtubule Motors
Jonathan W. Driver, Karthik Uppulury, Dale K. Jamison, Anatoly B. Kolomeisky, Michael R. Diehl.
Although the motions of many sub-cellular cargos are known to be driven by teams of microtubule motor proteins, understanding the role collective motor function plays in intracellular transport has been difficult to characterize. One confounding issue is that a team of molecular motors can adopt a spectrum of microtubule-associated configurations, depending on the number of bound motors and their organization on the microtubule. Since each microstate configuration can confer different mechanical and dynamic properties to a cargo, understanding how molecular motors function collectively ultimately requires knowledge of their relative contribution to cargo motility. To address this issue, we have developed methods to examine the load-dependent transport properties of structurally-defined multiple motor systems composed of two kinesin-1 molecules. Herein, we describe a discrete microstate transition-rate model of two-kinesin mechanics that can explain several unexpected behaviors observed in our assays. While this model accounts for a comprehensive spectrum of geometric arrangements of motors between a cargo and the microtubule, transition rates between microstate configurations are almost exclusively parameterized using data from single-kinesin optical trapping measurements. Overall, our model shows strong agreement with our data and recapitulates the central experimental finding that configurations where two kinesins both assume a portion of the applied load are rare and short-lived, causing two kinesins to exhibit negative cooperativity and properties that resemble the action of a single motor molecule. The bottom-up construction of our model also allows us to explore the effects of changes in single motor properties such as compliance, force-velocity relationship, and forward/backward stepping behavior on the dynamics of the system as a whole. This now provides a foundation for analyses of transport behaviors produced by more complex systems composed of different numbers, types and geometric arrangements of motors.

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Kinesin Generates a Nucleotide-Dependent Distortion of the Microtubule Lattice
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The kinesin molecular motors largely divide into two distinct functional forms: in the motile form, the energy of ATP hydrolysis powers travel along microtubules; in the depolymerizing form, ATP hydrolysis drives disassembly of microtubules. While significant advances have been made in the understanding structural basis of kinesin motility, the structural basis for ATP-driven depolymerization remains largely mysterious. We used high-resolution cryo-EM to visualize an unexpected, nucleotide-driven distortion of the microtubule structure when decorated by motile kinesins. This distortion is generated when ATP analogs bind in kinesin's active site and ''pinch'' two of kinesin's microtubule-binding domains together, changing the molecule's grip on the microtubule surface and leading to a relative rotation of alpha- and beta- tubulin subunits within the tubulin dimer. The tubulin subunit rotation is accompanied by a ~2% longitudinal contraction of the microtubule lattice. We observed this effect in the presence of two highly-divergent motile kinesins: ''conventional'' plus-end directed kinesin, as well as minus-end directed ncd. We infer that this functional behavior is likely a general feature of the kinesin family proteins, and may inform the mechanism of the depolymerizing kinesins.

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Electrostatically Biased Binding of Kinesin to Microtubules
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Molecular motors are the essential agents of movement within living organisms. Together with their cellular highways, microtubules and actin filaments, these proteins drive the beating of sperm, the division of cells and the muscular movement of organisms. We have established a multi-scale modeling approach to study fundamental details of motor function. The methods include bioinformatic analysis supplemented with all-atom molecular mechanics (to probe essential conformational changes) and coarse-grained Brownian dynamics (for diffusional encounters of motors with their respective track). As an example application, we describe results obtained for the kinesin-microtubule system.

Some kinesin molecular motors walk along microtubules, whilst others hop. In both cases, individual kinesin heads cycle on and off the microtubule. Recent evidence suggests that the on-reaction is directionally biased, by an as-yet unknown mechanism. Here we use atomistic Brownian dynamics simulations combined with real-world mutagenesis to show that incoming kinesin heads undergo electrostatically-guided diffusion-to-capture by microtubules, and that this produces directionally-biased binding. Kinesin-1 heads are initially rotated by the electrostatic field so that their tubulin-binding sites face inwards, and then steered towards a plus-endwards binding site. In tethered kinesin dimers, this bias is amplified, and in tethered kinesin-14, which is minus-end-directed, the bias is reversed. A 3-residue sequence (RAK) in kinesin helix alpha-6 is predicted to be important for electrostatic guidance. Real-world mutagenesis of this sequence powerfully influences kinesin-driven microtubule sliding, with one mutant producing a 6-fold acceleration over wild type.
Together these findings indicate that the adopted multi-scale modeling approach represents a promising strategy for designing motors with enhanced association rates via the tailoring of electrostatic interactions.

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Acetylation of Alpha Tubulin Lysine-40 Alone is not Sufficient for Changes in Kinesin-1 Motility
Neha Kaul, Virupakshi Soppina, Kristen J. Verhey, Edgar Meyhofer.
Kinesin-1 is a processive, plus end-directed motor that is responsible for major microtubule-based intracellular transport. It has been previously shown through *in vivo* studies that kinesin-1 preferentially translocates along certain subsets of microtubules, which are marked with specific posttranslational modifications (PTMs). We hypothesize that PTMs of tubulin directly influence the interaction of kinesin-1 with microtubules. In order to investigate the role of acetylation of α-tubulin at Lysine 40 (K40) in this context, we examined the binding affinity of kinesin in solution to acetylated and deacetylated microtubules in the presence of AMPPNP. We further characterized the single molecule motility properties of kinesin-1 on acetylated and deacetylated microtubules using Total Internal Reflection Fluorescence (TIRF) microscopy. To generate acetylated and deacetylated microtubules, purified bovine tubulin was treated with the enzymes MEC-17 and SIRT2, respectively. Kinesin-1 motors were either expressed in COS cells or purified from bacterial cells. We found that kinesin-1 shows similar binding affinity, velocity and run length on acetylated and deacetylated microtubules as measured in these *in vitro* assays. Our results suggest that an alteration in the state of acetylation of K40 on α-tubulin in the microtubules does not result in changes in the catalytic cycle and strong or weak-binding states of the motor. We conclude that kinesin-1 cannot directly recognize the presence of an acetyl group on K40 of α-tubulin and hence this modification alone is not sufficient to explain the preferential motility of kinesin-1 observed *in vivo*. Rather, K40 acetylation appears to mark a subset of microtubules with other structural or biochemical alterations that are recognized as trafficking cues by kinesin-1.

658-Pos Board B458
Characterization of the Plant-Specific Rice Kinesins
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Kinesin is a motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics and signal transduction. Kinesin converts chemical energy from ATP into mechanical force. Kinesin family is classified into some subfamilies. Some species of kinesin derived from vertebrate have been well studied. However, not so many studies for kinesins of plants have been done yet. Recently, the genome sequences of rice were completed. Bioinformatical analyses revealed that at least 41 kinesin-related proteins were encoded on the rice genome. In this study, we focused on the two rice kinesins; 1. O12 that has a calponin homology domain, 2. K23 that belongs to At1 subfamily in kinesin-7. The cDNAs of the kinesin motor domain was subcloned into expression vector pET and transformed into *E. coli* BL21 (DE3). kinesin motor domains were expressed and purified by Co-NTA column. The biochemical characterizations of the two rice kinesins were studied. The microtubule-dependent ATPase activity of the two rice kinesins motor domains were 30~60-fold lower than that of conventional kinesin. Kinetic analyses using stopped-flow demonstrated that ATP binding to O12 in the absence of microtubule was extremely slow compared with that of conventional kinesin. While, ATP binding to K23 was not accelerated by microtubule. Furthermore, interestingly ATPase activity of O12 in the absence microtubule regulated by actin. The O12-tail fused with GFP was observed to localize in the actin filament in the onion cell. The two plant specific rice kinesin O12 and K23 were shown to have unique enzymatic properties.